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Diamond based relaxometry for biosensing

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Chapter 5

General discussion

The detection of Free radicals with FNDs was not realized before 2020[1-4]. This unique technique allows the detection of free radicals in real-time for different applications. Firstly, the detection of free radical generation in a chemical reaction in a biological environment [2]. Second, quantifying intercellular free radical generation in a single living cell or even in specific organelles like mitochondria [5].

It is very important to find appropriate conditions to ingest FNDs inside the cell. Furthermore, it is crucial to evaluate FNDs retaining capacity inside the cells to measure free radical generation under shear stress condition, and targeting FNDs to a specific organelles like nuclei and mitochondria to measure organelle specific free radical generation. For this purpose, in this thesis, we optimized nanoparticle uptake environment for cells, overcome the challenge of free radical detection under shear stress condition in real time and detecting organelles specific free radical generation. To achieve this goal, we used relaxometry based on fluorescent nanodiamond particles (FNDs).

In chapter 2 we evaluated two types of environments: a confined microenvironment in a microfluidic chip and a macro environment in a Petri dish. We then observed under which conditions FNDs were ingested inside the macrophage cells. We used different geometries of microfluidic channels to create the microenvironment. Microenvironment primarily modifies the amount of CO_2 and the medium pH in addition to offering a very high surface area to volume (SAV) ratio. In this chapter we used 3 different cells macrophage j774, Hela and BSK 21. Depending on the morphology cells showed different uptake in the micro and macro environment. In the microenvironment nanoparticle uptake in macrophages was lower than in the macro environment. To investigate the uptake difference, we explored the effect of different factors such as CO_2 concentration in the medium, pH, and surface area to volume (SAV) ratio in the microfluidic chips. We also compared uptake differences in BHK21 and Hela cells. Finally, we modified channels and Petri dishes with gelatin.

We found that the pH inside the microfluidic channel was highly acidic whereas in a Petri dish and in a flow channel the pH was 8 and 8.3 respectively. On the other hand, Petri dishes that were kept overnight in a 15% and 5% CO_2 supplying incubator showed the pH values of 7.3 and 8.1 respectively. From the above PH values, it is confirmed that CO_2 accumulates in the microfluidic environment in the smaller medium volume, which reduces the extracellular pH and affects the nanoparticles uptake inside the cells.

Another assessment of surface area to volume ratio of channel (22 cm^2/mL) compared to the Petri dish (3.8 cm^2/mL) affect nanoparticles uptake in channels. To evaluate this, we incubated microfluidic volume of 113 µL FNDs suspension in the Petri dish instead of 500µL FNDs suspension. It was evident from the result uptake was higher in 113µL FNDs suspension rather than 500ul in petridish. This is the reason of small volume of Suspension cover only the glass bottom part of petridish rather than the plastic part of the petridish. In contrast, the same volume of suspension covered the whole microfluidic channel even in the inlet and outlet reservoirs.

Furthermore, Morphology of the cells affected the nanoparticle uptake. Macrophage J774 cells are circular while BHK21 and Hela cells are widely spread. This allowed us to verify the uptake in the BHK-21 cells is indeed influenced by larger surface coverage due to widely spread morphology. In the case of HeLa cells, the result is similar as for BHK-21 cells where uptake in cells cultured in the microfluidic devices was more than in a Petri dish.

The height of the cells in gelatin-coated channels was considerably higher than that in gelatin coated Petri dishes, which was similar to uncoated devices. However, the FNDs uptake in cells cultured in gelatin coated channels was higher than in gelatin coated Petri dishes.

From the above findings it is clear that nanoparticle uptake inside cells is not only affected by the cell culture environment but also the morphology of cells.

Chapter 3 Shear stress plays an important role for maintaining the vascular tone. Shear stress within the physiological range maintains the physiological function by balancing NO* and $O_2^{\cdot -}$ production, while very low and beyond the physiological range of shear stress increases free radical formation and causes pathological conditions.

We investigate radical formation in HUVECS in a microfluidic environment under different flow conditions typically found in veins and arteries. Here we monitored free radical generation before, during, and after flow conditions. We investigated whether shear stress has any influence on nanoparticle retaining inside the cells. To ensure the retaining capacity of HUVECs under flow conditions we quantified the number of nanoparticles in the static group and shear stress groups. We found that cells under shear stress in the physiological range or gradually increased shear stress retained an equal number of nanoparticles as in the static group. However, when cells suddenly were exposed to shear stress beyond the physiological range, they showed lower number intracellular nanoparticles than the static group. It is required for relaxometry to retain FNDs inside the cells even under conditions out of physiological range of shear stress.

For free radical measurements, HUVECs cells were exposed to shear stress for a short term 30 mins and long term 4 hrs. When exposed to shear stress in the venous and low atrial range free radical production increased inside the cell. A DAF FM assay was used to evaluate the NO* production and the cell morphology was analyzed to measure fiber and nuclear alignment with the flow direction. Under these flow condition, cells produce more NO*, aligned with the flow direction and maintain a tight junction with each other. While sudden exposure to high shear stress (20dyn/cm^2) caused HUVECs cell damage or death. As a result, the cells failed to produced free radicals and increased the T1. Further analysis with confocal microscopy showed that fibers and the nucleus were perpendicular with the flow direction. Under this flow condition, cells did not form tight junctions or bonding with each other.

However, for long term experiments when shear stress gradually increased after every 30 mins from the venous to the arterial range, cells gradually adapted to the shear stress and increased NO* production. Confocal images proved that fibers and nuclei were aligned with the flow direction. Cells formed tight junctions with each other. Intracellular NO* was quantified from a standard curve, which was prepared by using known concentrations of Spermine NONOate. Likewise, the intracellular superoxide level was quantified from the standard curve. Defined concentrations were prepared by using the chemical reaction of Xanthine and Xanthine peroxidase.

Furthermore, the NO* generation range in HUVECs cells was between 0.1 to 279 µM while the superoxide production range was between 0.0001 to 3 µM. However, in this chapter the superoxide production is very low compared to NO* production in different shear stress conditions. As a result, T1 showed good agreement with NO* formation.

Chapter 4 Free radical generation specifically in different organelles is still unknown. There is no device, which can detect free radical generation in different organelles directly. FNDs responses to magnetic field from a single electron spin from 51 to 10 nm [6]. As a result, it is very important to control the location and target FNDs to specific organelles of a cell. In this chapter we measured free radical generation in the cytosol, mitochondria and the nucleus by targeting FNDs to these organelles. Different types of nanoparticles such as bare diamond (FNDs), Anti VDAC2 antibody coated FNDs (MIT-FNDs) and SV40 NLS conjugated FNDs (NLS-FNDs) were used for measurements in the cytoplasm, mitochondria and the nucleus respectively. Different doses of Acetaminophen were used to generate cellular toxicity. An overdose of APAP can lead to acute liver poisoning and death [7,8]. Physiologically, APAP is metabolized in the liver, by cytochrome P450s [9-11] of the active metabolite N-acetyl-P-benzoquinone imine (NAPQI) which is efficiently detoxified by conjugation with glutathione [12,13]. However, higher doses of APAP lead to critical depletion of glutathione and oxidative stress. This oxidative stress amplifies the formation of free radical causing the cessation of mitochondrial adenosine triphosphate (ATP) synthesis and nuclear deoxyribonucleic acid (DNA) fragmentation. Together, the nuclear DNA damage and the extensive mitochondrial dysfunction result in necrotic cell death.

In this chapter we confirm the presence of FNDs in the target organelles by using Mander's coefficients. Relaxation time was measured to detect free radical generation in the cytosol, mitochondria and the nucleus. Free radical generation was varied with different time points with different organelles. In cytosol free radical generation was observed at 18 h treatment with higher dose 2 and 4 mM. Whereas, in nucleus and mitochondria it started after 3 hrs of APAP treatment. The quantity of free radical generation was dependent on APAP dose, treatment duration and location of measurement. In mitochondria higher doses of APAP induced free radical generation and

cessation earlier compared to the lower concentrated drug. On the other hand, in the nucleus free radical generation was observed faster after 3 hrs of treatment among all drug treatment groups. Free radical generation was evaluated by conventional methods like the DCFDA and the DHE assay. DCFDA only detected free radical generation at the higher dose treatment. On the other hand, the DHE assay detects intracellular super oxide. In this chapter the DHE assay showed superoxide generation among all-time points and all organelle groups. Additionally, nuclear morphology analysis showed significant differences only after 18h of treatment. The MTT assay showed significantly reduced cellular metabolic activity in the 18 h treatment group.

From the above discussion, it is evident that relaxometry can distinguish organelle-specific free radical generation while conventional methods reveal the whole cell's free radical generation.

In the future, we believe that NV-based T1 relaxometry will be one of the most powerful tools to detect radicals in biological systems. Therefore, more knowledge needs to be gained on how different environmental features influence ND quantum sensors to clearly determine the impact of one parameter on biological processes.

1 Barton J, Gulka M, Tarabek J, Mindarava Y, Wang Z, Schimer J, Raabova H, Bednar J, Plenio MB, Jelezko F, Nesladek M, Cigler P. Nanoscale Dynamic Readout of a Chemical Redox Process Using Radicals Coupled with Nitrogen-Vacancy Centers in Nanodiamonds. ACS Nano. 2020 Oct 27;14(10):12938-12950. doi: 10.1021/acsnano.0c04010. Epub 2020 Aug 19. PMID: 32790348..

2. Perona Martínez F, Nusantara AC, Chipaux M, Padamati SK, Schirhagl R. Nanodiamond Relaxometry-Based Detection of Free-Radical Species When Produced in Chemical Reactions in Biologically Relevant Conditions. ACS Sens. 2020 Dec 24;5(12):3862-3869. doi: 10.1021/acssensors.0c01037. Epub 2020 Dec 3. PMID: 33269596; PMCID: PMC8651177.

3. Sharmin R, Hamoh T, Sigaeva A, Mzyk A, Damle VG, Morita A, Vedelaar T, Schirhagl R. Fluorescent Nanodiamonds for Detecting Free-Radical Generation in Real Time during Shear Stress in Human Umbilical Vein Endothelial Cells. *ACS Sensors. 2021*; *6*(12): 4349-4359. https://doi.org/10.1021/acssensors.1c01582

4. Nie L, Nusantara AC, Damle VG, Baranov MV, Chipaux M, Reyes-San-Martin C, Hamoh T, Epperla CP, Guricova M, Cigler P, van den Bogaart G, Schirhagl R. Quantum Sensing of Free Radicals in Primary Human Dendritic Cells. Nano Lett. 2022 Feb 23;22(4):1818-1825. doi: 10.1021/acs.nanolett.1c03021. Epub 2021 Dec 20. PMID: 34929080; PMCID: PMC8880378.

5. Nie L, Nusantara AC, Damle VG, Sharmin R, Evans EPP, Hemelaar SR, van der Laan KJ, Li R, Perona Martinez FP, Vedelaar T, Chipaux M, Schirhagl R. Quantum monitoring of cellular metabolic activities in single mitochondria. Sci Adv. 2021 May 19;7(21):eabf0573. doi: 10.1126/sciadv.abf0573. PMID: 34138746; PMCID: PMC8133708.

6. Grinolds. M. S, Hong. S, Maletinsky. P, Luan. L, Lukin. M. D, Walsworth. R. L, Yacoby. A. Nanoscale magnetic imaging of a single electron spin under ambient conditions. Nat. Phys. 2013, 9 (4), 215−219.

7. Litovitz TL, Klein-Schwartz W, Rodgers GC Jr, Cobaugh DJ, Youniss J, Omslaer JC, May ME, Woolf AD, Benson BE. 2001 Annual report of the American Association of Poison Control Centers Toxic Exposure Surveillance System. Am J Emerg Med. 2002 Sep;20(5):391-452. doi: 10.1053/ajem.2002.34955. PMID: 12216043.

8. Lazerow SK, Abdi MS, Lewis JH. Drug-induced liver disease 2004. Curr Opin Gastroenterol. 2005 May;21(3):283-92. doi: 10.1097/01.mog.0000160043.10804.60. PMID: 15818148.

9. Chen W, Koenigs LL, Thompson SJ, Peter RM, Rettie AE, Trager WF, Nelson SD. Oxidation of acetaminophen to its toxic quinone imine and nontoxic catechol metabolites by baculovirus-expressed and purified human cytochromes P450 2E1 and 2A6. Chem Res Toxicol. 1998 Apr;11(4):295-301. doi: 10.1021/tx9701687. PMID: 9548799.

10. Patten CJ, Thomas PE, Guy RL, Lee M, Gonzalez FJ, Guengerich FP, Yang CS. Cytochrome P450 enzymes involved in acetaminophen activation by rat and human liver microsomes and their kinetics. Chem Res Toxicol. 1993 Jul-Aug;6(4):511-8. doi: 10.1021/tx00034a019. PMID: 8374050.

11. Nelson SD. Molecular mechanisms of the hepatotoxicity caused by acetaminophen. Semin Liver Dis. 1990 Nov;10(4):267-78. doi: 10.1055/s-2008-1040482. PMID: 2281334.

12. Hinson JA, Reid AB, McCullough SS, James LP. Acetaminophen-induced hepatotoxicity: role of metabolic activation, reactive oxygen/nitrogen species, and mitochondrial permeability transition. Drug Metab Rev. 2004 Oct;36(3-4):805-22. doi: 10.1081/dmr-200033494. PMID: 15554248..

13. Reid AB, Kurten RC, McCullough SS, Brock RW, Hinson JA. Mechanisms of acetaminophen-induced hepatotoxicity: role of oxidative stress and mitochondrial permeability transition in freshly isolated mouse hepatocytes. J Pharmacol Exp Ther. 2005 Feb;312(2):509-16. doi: 10.1124/jpet.104.075945. Epub 2004 Oct 1. PMID: 15466245.